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ABSTRACT

This review is intended to assess available literature in order to define the range of nitrate/nitrite effects on animals. Though the literature deals primarily with livestock and experimental animals, much of the contemporary research is concerned with human nitrite intoxication. Thus, the effects on man are discussed where appropriate. Some of the aspects of nitrate/nitrite toxicity that are discussed are: (1) factors affecting accumulation in plants and in water; (2) factors affecting toxicity in animals including humans; and (3) both acute and sublethal toxicity. Toxicity of nitrogen oxides and nitros amines are discussed as well as limits of nitrate/nitrite ingestion for humans in both food and water. This literature review includes a bibliography and a summary and conclusions section. Among the conclusions are: (1) the nitrate content of water is a matter for public concern because of the potential health hazard for infants; (2) human infants are far more susceptible than adults to methemoglobinemia; (3) there is little evidence to indicate that nitrosamines are more than a potential hazard to animal or human health; and (4) nitrate toxicity may occur when animals consume forages that have accumulated nitrates as a result of environmental factors. (MR)

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Nitrates, Nitrites, And Health

BARBARA S. DEEB AND KENNETH W. SLOAN

Bulletin 750
Agricultural Experiment Station
Colleges of Agriculture and Veterinary Medicine
University of Illinois at Urbana-Champaign

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SINCE THE TIME OF N. S. MAYO'S ACCOUNT of "cornstalk poisoning," the narrative that begins the next section, there have been various investigations of nitrates and nitrites and their effects on many species. During the past 20 years attention has been focused on possible chronic toxicity — that is, the sublethal effects of long-term ingestion of low levels of nitrate.

This review is intended to assess available literature in order to define the range of nitrate/nitrite effects on animals. Though the literature deals primarily with livestock and experimental animals, much of the contemporary research is concerned with human nitrite intoxication; thus, the effects on man are discussed where appropriate.

The following publications are suggested as supplemental materials for those who wish more detailed information on specific topics than will be presented here. Complete citations are given in the bibliography.

1. D. H. K. Lee (1970). "Nitrates, nitrites, and methemoglobinemia." A comprehensive review and bibliography concerning nitrate/nitrite intoxication in man.

2. M. J. Wright and K. L. Davison (1964). "Nitrate accumulation in crops and nitrate poisoning in animals." An excellent review.

3. J. J. Hanway *et al.* (1963). "The nitrate problem." A review including information on tests for nitrate in plants and water.

4. H. S. McKee (1962). *Nitrogen metabolism in plants.* Nitrate physiology in plants.

5. A. Nason (1962). "Symposium on metabolism of inorganic compounds. II. Enzymatic pathways of nitrate, nitrite, and hydroxylamine metabolism

6. F. G. Viets, Jr., and R. H. Hageman (1971). "Factors affecting the accumulation of nitrate in soil, water, and plants." A review.

7. National Academy of Sciences (1972). *Accumulation of nitrate.* An examination of problems associated with accumulation of nitrate nitrogen and related nitrogenous compounds in the environment.

8. R. J. Emerick (1974). "Ecological problems of high level nutrient feeding." Review of impact of animal wastes from intensive feeding operations.

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HISTORICAL BACKGROUND

> On April 3rd, 1895, in the capacity of veterinarian to the State Live Stock Sanitary Commission, I was called to Winfield, Kansas, to investigate a disease which had caused the death of ten head of cattle out of a herd of thirty-four. The cattle were mixed native cattle, thin in flesh. They had been running on pasture land and had been fed some hay. They were watered from a well and windmill but the supply of water available for the cattle appeared limited. The owner of the cattle first noticed some of the cattle sick about noon, on April 1st, they having previously been fed some corn fodder scattered in the corral. In eighteen hours after the first animal was taken sick, six were dead and six more sick. Two of the sick animals recovered. The symptoms as described by the owners were as follows: The animals appeared stupid and sometimes delirious. There was trembling of the voluntary muscles with a reeling, staggering gait, the animals appearing weak in the hind quarters. The animals urinated frequently and the bowels were loose. When the animals fell down they would not attempt to rise and would die in a short time.

N. S. Mayo's 1895 account of "cornstalk poisoning" is considered the earliest detailed report of nitrite toxicity. Mayo reported several similar cases, witnessed by himself or his colleagues, in which he associated the "poisoning" with the cattle's ingestion of green corn fodder. He observed that leaf axils and stalks from corn plants similar to those eaten by the cattle contained abundant crystals of potassium nitrate. After analysis of some stalks indicated that they contained 18.3 percent KNO_3, or 2.5 percent nitrate nitrogen (NO_3-N), dry weight, Mayo administered KNO_3 to three cattle: one received 200 g in feed, one was given 300 g as a drench, and one was given 500 g as a drench. The latter two died with signs similar to those of the cattle described earlier. He concluded (Mayo, 1895) that these were "symptoms of potash poisoning."

The nitrate ion was later identified as the toxic component of nitrate salts when researchers were studying outbreaks of "oat hay poisoning" in Wyoming (Bradley *et al.*, 1939; 1940). Analysis of oat hays from stacks that had been used as a source of feed during large-scale losses of livestock revealed that the NO_3-N content of the stalks and leaves was about 0.3 to 1.0 percent. Three calves were given a water extract of the oats, a KNO_3 solution of comparable strength, or an oat extract from which 70 percent of the nitrate had been removed by crystallization. The calf receiving the denitrated extract survived; the others died with signs of "oat hay poisoning." The affected animals had high methemoglobin levels in their blood (Bradley *et al.*, 1940).

The role of nitrate in "cornstalk poisoning" and "oat hay poisoning" was clarified by Davidson *et al.* (1941), who showed that the nitrate

that accumulated in the plants was reduced to nitrite after ingestion. The nitrite was absorbed into the bloodstream, where it reacted with hemoglobin to form methemoglobin. Signs of hypoxia developed when methemoglobin levels were sufficiently high.

In 1946 swine were found to be affected by nitrites when Gwatkin and Plummer produced signs of toxicity in fasted pigs by administering orally approximately 1 g KNO_3/lb body weight (300 mg/kg NO_3-N). The pigs died within 24 hours from severe gastritis; methemoglobinemia was not observed. Oral doses of KNO_2 (approximately 40 to 90 mg/kg NO_2-N) caused death from methemoglobinemia within 65 minutes when given to fasted pigs but was not fatal to normally fed pigs.

A case of naturally occurring poisoning in swine was traced to the feeding of refuse meat cooked in well water (Winks *et al.*, 1950). Analysis of the resultant soup revealed a sodium nitrite ($NaNO_2$) content of up to 1,127 parts per million (ppm), equivalent to 225 ppm NO_2-N. Subsequent feeding trials showed that 90 mg of $NaNO_2$ caused deaths in mature fasted swine when given as a 1-percent solution.

Considerable concern about nitrate/nitrite ingestion developed in 1945 when Comly described methemoglobinemia in human infants given formulae prepared with well water of high nitrate content. Additional reports from the United States and Europe followed rapidy, and foods with high nitrate content were also implicated (Lee, 1970). By the 1950's nitrate/nitrite ingestion through food, feed, and water had been clearly recognized as potentially hazardous for man and livestock.

METHODS OF EXPRESSING NITRATE/NITRITE VALUES

Researchers have used a variety of methods to express the nitrate/nitrite content of feeds and water and of substances administered to determine resulting effects on animals. Hanway *et al.* (1963) compiled a list of the most common methods for designating nitrate content of feeds and water and the factors for converting from one designation to another. These factors are presented in Table 1.

In this review, the form of expression used in cited publications is presented, followed by conversion to a standard expression of nitrate nitrogen (NO_3-N). Content of nitrate or nitrite in feeds or tissues is given as percent NO_3-N or, sometimes, as parts per million (ppm) NO_3-N; for water the conversion is to milligrams per liter (mg/l) NO_3-N. Where sufficient information is available, the level of nitrate or nitrite administered experimentally to animals is presented as milligrams per kilograms (mg/kg) of body weight.

Table 1. Methods of expressing nitrate and nitrite contents of feeds and water, atomic weights of the various substances, and conversion factors (after Hanway et al., 1963)

Nitrogenous substance	Chemical formula or designation	Atomic, molecular, or ionic weight	Multiplication factors for converting from nitrogenous substance in column 1 to[a]			
			N	NO_2	NO_3	KNO_3
Nitrate nitrogen	NO_3-N	14	1	3.3	4.4[b]	7.2
Nitrite nitrogen	NO_2-N	14	1	3.3	4.4[b]	7.2
Nitrite	NO_2	46	0.30	1	1.3	2.2
Nitrate	NO_3	62	0.23	0.74	1	1.6
Sodium nitrate	$NaNO_3$	85	0.16	0.54	0.73	1.2
Potassium nitrate	KNO_3	101	0.14	0.46	0.61	1

[a] Examples (percent, mg/l, or ppm):
1.0 NO_3-N = 4.4 NO_3 or 7.2 KNO_3
1.0 NO_3 = 0.23 NO_3-N or 1.6 KNO_3
1.0 KNO_3 = 0.61 NO_3 or 0.14 NO_3-N

[b] For convenience this factor is sometimes rounded to 4.5.

NITRATE ACCUMULATION

Factors Affecting Accumulation in Plants

Nitrates represent an essential step in the incorporation of nitrogen from the soil into plant protein. Most of the nitrogen absorbed by plants is in the form of nitrate, which is usually transported and assimilated so rapidly within the plant tissues that normal concentrations rarely exceed a few hundred parts per million. Under some circumstances, however, excessive nitrates accumulate in plants, and these plants may be toxic to an animal that ingests them.

Plant-associated factors that influence nitrate accumulation include plant species variability, selective localization within the plant, stage of maturity, and nitrate reductase activity. Environmental factors associated with nitrate accumulation include drought, high temperature, cloudy weather, low availability of nutrients, damage by insects and herbicides, and excessive soil nitrogen (Dodd, 1966; Lee, 1970; McKee, 1962; Nason, 1962; Viets and Hageman, 1971; Wolff and Wasserman, 1972; Wright and Davison, 1964).

Soil application of nitrogen fertilizers is the factor most easily controlled and most widely blamed for nitrate accumulation in plants and water. Recently, efforts have been made to determine whether plants grown on heavily fertilized soil are more likely to be toxic to

livestock; some of these experiments are described below. To date, no positive correlations have been found between excess nitrogen fertilization of soils and poor performance by animals.

For three and a half months, cattle were fed an exclusive diet of forages (hay of fodder barley) from plots treated with ammonium nitrate at 0, 400, 600, 800, or 1,000 kg/hectare. Although the forage contained supposedly toxic levels of nitrate and nitrite, sampling of hemoglobin and methemoglobin did not reveal significant changes that could be related to fertilization rates. Serum nitrate levels were slightly elevated (Buruiana *et al.*, 1969).

When nitrogen was applied as calcium ammonium nitrate to pasture grasses periodically throughout the grazing season at levels of 0, 184, or 368 lb/A (0, 83.4, or 166.9 kg/A), nitrate levels in the grass increased with each nitrogen application. Highest accumulation occurred near the end of the grazing season. Sheep grazing on the pastures increased performance with the first and second applications of 184 pounds. No significant detrimental effect on the sheep was observed. It was suggested that levels of nitrogen fertilizer, up to the maximum used in the study, could be applied uniformly to pasture throughout the grazing season without adverse effect on sheep performance (O'Donovan and Conway, 1968).

In another feeding trial, ewes fed high-nitrate forages containing up to 1.3 percent nitrate (0.29 percent NO_3-N) from land fertilized with 1,000 lb/A ammonium nitrate failed to show performance significantly different from that of controls (Davison, McEntee, *et al.*, 1964).

O'Donovan and Conway (1968) stated that, apparently, as far as pastures and forages are concerned, "decreasing returns from nitrogen fertilization may become a reality before dangerous nitrate levels are reached."

Postharvest Nitrate Reduction in Feeds

Nitrate reduction in feeds and foods after harvest, caused by naturally occurring microbial contaminants, may represent a matter of more direct concern to growers and livestock producers. Accumulations of nitrite in feeds could significantly increase the likelihood of their toxicity.

Postharvest conversion of nitrate to nitrite has been demonstrated by many (Wright and Davison, 1964). Jones and ap Griffith (1965) noted that retrospective studies of the early reports of oat hay poisoning in the United States indicated that, in most of the outbreaks, the hay

was damp when fed. This suggested the possibility that some of the nitrate in the hay may have been reduced to nitrite prior to feeding. Several earlier studies supported this conclusion (Olson and Moxon, 1942; Davidson *et al.*, 1941; Riggs, 1945; Barnett, 1953; Gilbert *et al.*, 1946). Further, Jones and ap Griffith confirmed the reduction of nitrates to nitrite in moist hay by showing that, after three days' incubation, 15 to 43 percent of added nitrate could be recovered as nitrite. They suggest that discrepancies in the literature relating to toxic levels of nitrates in feeds may be the result of neglecting this consideration.

Nitrates are readily reduced in silage, forming nitrite and the dangerous "silo gases," N_2O, NO, NO_2, and N_2O_4. Silages of high moisture content may lose a higher percentage of nitrate through the gaseous denitrification process than do low-moisture silages. Hydrogen ion concentration also influences denitrification: The greatest nitrate losses occur when pH does not fall below 5.5 (Jacobson and Wiseman, 1963). Besides formation of the toxic products, nitrate reduction in silage may have a more subtle effect in the possibility of carotene destruction by nitrite, nitric oxide, and nitrogen tetroxide in an acid medium (Olson *et al.*, 1963).

Factors Affecting Nitrate Accumulation in Water

Increased nitrate levels in ground water or in rural and municipal water supplies usually occur as a result of contamination by organic wastes from humans or animals or by nitrogenous fertilizers. Excessive levels of nitrates have been found in rural wells in many areas (Singer, 1968; Lee, 1970) and in a number of municipal water supplies (American Water Works Association, 1967).

In some agricultural areas, the presence of nitrates in ground water is widespread; moreover, nitrate concentrations may be expected to increase as farmers continue to use nitrogenous fertilizers to improve crop yields. According to a report of the U.S. Public Health Service (1962), some municipal wells in agricultural areas of southern California deliver water with nitrate levels in excess of 92 ppm NO_3 (20.8 ppm NO_3-N), more than twice the permissible level of 45 ppm NO_3 (10 ppm NO_3-N).

Analysis of 5,000 water samples in Missouri showed that 42 percent contained over 5 ppm NO_3-N, with a high correlation between proximity to livestock areas and concentrations of nitrates and nitrites in wells. The results of analyses of soil samples revealed that concentrations of nitrates 5 feet below the surface were maintained for many

years. In some areas wells as deep as 100 to 300 feet contained significant levels of nitrates. It was believed that nitrates were leached from manure at the surface and were carried into the underground water sources. No instances were found where nitrates in water could be traced to chemical nitrogen fertilizer applied to crops or to contamination from fertilizer storage. Moreover, samples of pond water contained low concentrations of nitrate despite runoff from pastures and feeding areas; nitrate ions in runoff were assumed to be utilized by the aquatic plants (Smith, 1965).

In Illinois only 4 of 789 public ground water supplies were found to contain more than 45 ppm nitrate in 1969. However, several thousand domestic and farm wells may contain more than the recommended level (Walker, 1969). An Illinois river traversing heavily fertilized farmland was found to contain between 19 and 56 ppm nitrate (Larson and Larson, 1967). Reports from Canada, England, and continental Europe indicate a similar situation in other countries (Lee, 1970).

The process by which nitrates may be leached from manure accumulated in livestock production was demonstrated by Gillham and Webber (1969), who measured nitrogen contribution to ground water by leachates from a barnyard. Average nitrogen concentrations were measured in ground water before (2 ppm) and after (5 to [illegible] ppm) it had passed beneath the barnyard. Approximately 90 percent of the nitrogen was in the nitrate form.

A study of ground water pollution in the Platte Valley in Colorado indicated that although water under feedlots contained much larger amounts of nitrate per unit area, irrigated farmland contributed more total nitrate to the ground water (Stewart *et al.*, 1967).

Volatilization of nitrogenous gases from animal wastes may also contribute to soil and water contamination. Based on measurements at Colorado's Seeley Lake, 2 km from a large cattle feedlot, enough ammonia was absorbed from the air in one year to raise the nitrogen concentration of the lake by 0.6 ppm. Although the ammonia content of precipitation appeared to increase in close proximity to the large feedlot, the amount of ammonia washed from the atmosphere by precipitation appeared to be insignificant when compared to the amount absorbed directly from the air by the surface of the lake (Hutchinson and Viets, 1969).

Other aspects of water contamination related to cattle feedlots and animal wastes were discussed at a conference sponsored by the Federal Water Pollution Control Administration (1970).

FACTORS AFFECTING TOXICITY OF NITRATE/NITRITE IN ANIMALS

Nitrate Reduction

The term "nitrate toxicity," used so frequently in the literature, is actually a misnomer. When found at normal levels in food and feed, nitrates are toxic only under conditions in which they are reduced to nitrites (Wolff and Wasserman, 1972). Experiments with dogs (Greene and Hiatt, 1954) demonstrated that relatively large doses of NO_3-N given as sodium nitrate could be administered orally (280 mg/kg body weight) or intravenously (570 mg/kg body weight) over several weeks without evidence of methemoglobinemia, altered blood pressure, or marked changes in renal hemodynamics. Approximately 90 percent of the nitrate dose was recovered in the urine. High tolerances of sheep to nitrates given intravenously (Pfander *et al.*, 1957) and of rats and rabbits to nitrates given orally (Kilgore *et al.*, 1959) have substantiated the conclusion that the nitrate ion as such is not responsible for the signs of "nitrate toxicity." It is, rather, the reduction of nitrate to nitrite that constitutes the hazard to animals. The ability to reduce nitrate is a characteristic of anaerobic and facultatively anaerobic microorganisms, especially the Clostridia and the Coliforms. Conversion of relatively nontoxic nitrate to toxic nitrite readily occurs in bacterially contaminated food or water or in gastrointestinal contents.

Both nitrate and nitrite ions are readily and passively absorbed into the bloodstream from the gastrointestinal tract. Bernheim and Dixon (1928) showed that nitrate may be converted to nitrite by liver enzymes of many animals, including livestock, and by striated muscles of rats and guinea pigs. Reduction in the liver must be slow, since other workers have not been able to produce methemoglobinemia by giving nitrates intravenously to animals (Greene and Hiatt, 1954; Pfander *et al.*, 1957). Although most nitrate is excreted by the kidney, some is recycled from the bloodstream back into the gastrointestinal tract by salivary and gastrointestinal secretions (Bloomfield, Hersey, *et al.*, 1962; Kearley *et al.*, 1962).

The studies of Wang *et al.* (1961) elucidated the metabolic fate of N^{15}-labeled potassium nitrate in cattle. Nitrate, nitrite, and ammonium ions were absorbed directly from the rumen. Nitrate and nitrite reached their highest blood levels 5 to 6 hours after rumen inoculation; ammonium levels were highest at 3 to 4 hours after inoculation. Curves for the excretion of N^{15} lagged behind curves for the appearance and disappearance of N^{15} in the blood by approximately 1 hour. Methemo-

globin formation closely followed the time course of nitrite formation in the rumen, indicating that the nitrite passed directly and rapidly into the blood. There was no evidence of conversion of nitrate to nitrite in the blood.

Hydroxylamine (NH_2OH) was suggested as another product of microbial reduction of nitrate in the rumen (Lewis, 1951). Winter's (1962) attempts to demonstrate hydroxylamine in blood of heifers following oral treatment with sodium nitrate were inconclusive. He was able to show that hydroxylamine and nitrite were rapidly reduced when added to bovine blood *in vitro* and suggested that this might account for the low level or absence of these substances in his *in vivo* experiments.

Smith and Layne (1969) confirmed the ability of hydroxylamine to produce methemoglobinemia in mice and found this compound to be more acutely toxic than sodium nitrite when each was given intraperitoneally. When given in equal sublethal doses, the two generated equivalent peak levels of methemoglobin, but the effects from hydroxylamine occurred much more rapidly than those from nitrite.

Reactions of Nitrite With Hemoglobin

Once in the blood, nitrite oxidizes the ferrous iron of hemoglobin to the ferric iron of methemoglobin, a brownish pigment incapable of transporting or releasing oxygen to the tissues.

According to Sunderman and Sunderman (1964), the small amounts of methemoglobin normally produced (0.03 to 0.13 g/100 ml) are readily reconverted to hemoglobin by the reducing action of methemoglobin reductase (termed diaphorase by Ross, 1963). If the capacity for reconversion is exceeded, abnormal concentrations will build up. Transformation of 70 percent of hemoglobin to methemoglobin is likely to be rapidly fatal (Lee, 1970).

In studies with blood from man and various livestock species, Smith and Beutler (1966) found that the rate of methemoglobin formation is related to methemoglobin reduction such that a rapid rate of formation is offset by a rapid rate of reduction. Quantitative relationships of methemoglobin formation were studied by Bartík *et al.* (1965) and by Bartík and Kupka (1967), who found that sodium nitrite produced less methemoglobin when used *in vivo* than when it was tested in blood in an *in vitro* system. This difference was attributed to protective factors in circulating blood that operate to maintain low levels of methemoglobin.

Nitrite may react with hemoglobin and methemoglobin molecules in other ways besides oxidation of ferrous hemoglobin. Smith (1967) pre-

sented evidence of the formation of a reversible complex between excess free nitrite and ferric heme groups of methemoglobin. Existence of this complex in nitrated erythrocytes can lead to an underestimation of methemoglobin levels by spectrophotometry. Such a complex probably exists only transiently *in vivo* but may contribute to the unusually sustained methemoglobinemia that nitrite produces in mice. Evidence obtained by Uchida and Klapper (1970) supported their conclusion that nitrite binds to the iron of methemoglobin and concomitantly reacts irreversibly with either the apoprotein or the porphyrin group without causing major disturbance of the protein structure. Rein *et al.* (1968) reported that, along with methemoglobin, nitrosohemoglobin is formed *in vitro* when nitrite is added to blood. The amount of nitrosohemoglobin depends upon the animal species but is especially high in species with actively metabolizing red blood cells. *In vivo*, under conditions corresponding to "sublethal nitrite poisoning" and in which the formation of nitrite-methemoglobin is excluded, nitrosohemoglobin still forms, and conversion to nitrosomethemoglobin may follow. Only a small part of the nitrosomethemoglobin is converted to nitrosohemoglobin by enzymatic reduction; the greater part decomposes to methemoglobin and nitrous oxide. The relative significance of this alternate means of methemoglobin formation with regard to age, health status, and species variability needs clarification.

Effects on the Circulatory System

Nitrite and organic nitrates have long been known as vasodilators when administered to monogastric animals (Hueper and Landsberg, 1940; Rath and Krantz, 1942). Sodium nitrite administered to dogs has been shown to reduce resistance in both large and small arteries, to lower systemic arterial pressure and total peripheral resistance, and to transiently increase heart rate and decrease myocardial contractile force (Rubin *et al.*, 1963). Swine given 20 mg/kg sodium nitrite (4 mg/kg NO_3-N) intravenously in a 10-percent solution showed a drop in blood pressure from 130 to 45 or 50 mm Hg. The effects of nitrate on blood pressure may, however, be influenced by several factors: the route of administration, the dosage, the duration of treatment, and the cation that accompanies the nitrate. Fregly (1964) found that rats developed hypertension when hypertonic sodium nitrate solutions were offered as the sole drinking fluid. The threshold concentration for elevation of blood pressure was between 0.15 and 0.175 M.

Calves given sodium nitrite intravenously showed a transitory fall in blood pressure during administration, with an upward adjustment

after the nitrite was injected (Asbury and Rhode, 1964). Wright and Davison (1964) concluded, from data not previously published by them, that the blood pressure of cattle fed 100 to 150 mg/kg NO_3-N daily did not differ from control animals. It appears that vascular collapse in cattle does not play a significant role in nitrite toxicosis; in monogastric animals, however, the effect of nitrite on the vascular system should be considered in cases of acute poisonings.

Temporal Relationships Between Administration of Nitrate or Nitrite and Development of Methemoglobinemia

Many experimentally induced nitrite intoxications in livestock have provided information regarding the temporal relationships among nitrate or nitrite concentrations in the blood, formation of methemoglobin, and development of signs of acute toxicity.

Diven, Reed, *et al.* (1962) administered sodium nitrate intraruminally to mature sheep. The magnitude of response varied directly with the dosage level of nitrate, and blood nitrate and methemoglobin reached their maximum levels between 4 and 6 hours after treatment. Signs of nitrite poisoning were most obvious between 6 and 8 hours after treatment. When sheep were similarly dosed with potassium nitrite, plasma nitrite rose to a peak within 2 hours, accompanied by progressive methemoglobinemia (Sinclair and Jones, 1967). Signs of toxicity generally occur in sheep when 10 to 20 percent of the hemoglobin has been converted to methemoglobin. Diven *et al.* (1964) administered potassium nitrite intraperitoneally and found that deaths in mature ewes occurred when methemoglobin levels exceeded 80 percent. Peak methemoglobin levels were higher and appeared earlier with increasing dose level. Similar results have been reported for cattle (Davison *et al.*, 1962; Winter, 1962).

In swine and dogs, maximum methemoglobin concentrations have been shown to occur 2 to 3 hours after oral or subcutaneous dosing with nitrite (Michalski, 1963; Sleight *et al.*, 1972). Signs of methemoglobinemia have appeared when 20 percent of the hemoglobin existed as methemoglobin. Oral administration of nitrite to swine caused acute toxic signs when 76- to 82-percent methemoglobin levels were reached. Deaths occurred 90 to 150 minutes after dosing (London *et al.*, 1967; Curtin and London, 1966).

Chicks fed nitrite did not show maximum methemoglobin levels until approximately 8 hours after initial ingestion of high-nitrite feed. Blood nitrite levels peaked about 24 hours after feeding began (Sell *et al.*, 1963).

In general, nitrite and methemoglobin peaks appear within 8 hours after animals receive large single doses of nitrite. Signs of methemoglobinemia appear when methemoglobin concentration reaches 20 percent; deaths occur at levels of 70 to 90 percent. It must be noted, however, that while blood nitrite and methemoglobin levels may be related in any one animal, methemoglobin production from identical doses varies from animal to animal (Winter, 1962).

Susceptibility to Methemoglobinemia

Species variation due to method of gastrointestinal nitrate reduction. The difference in nitrate susceptibility from one animal species to another is due primarily to anatomical and physiological differences in the gastrointestinal tract. The rumen of cattle and sheep contains a resident population of microbial flora that benefit their host by secreting cellulose-digesting enzymes. Ruminal contents are normally maintained at near neutral pH. In contrast, the simple stomach of monogastrics normally contains few bacteria because of hydrochloric acid secretion.

Nitrate reduction to nitrite and ammonia within the gastrointestinal tract is attributed solely to microorganisms (Lewis, 1951). In ruminants this reduction occurs primarily in the rumen (Pfander *et al.*, 1957; Wang *et al.*, 1961), whence the nitrate, nitrite, and ammonium ions are absorbed directly into the blood. Nicholas (1959) discussed the stepwise inorganic conversion of nitrate to ammonia and noted that in plants several metalloflavoprotein enzymes were involved in the process illustrated below.

metal requirement:		Mo $2e^-$	CuFeMg $2e^-$	CuFeMg $2e^-$	MnMg $2e^-$
oxidation-reduction state of N:	nitrate	nitrite	nitrogen dioxide	amine	ammonia
	NO_3	$\longrightarrow NO_2$	$\longrightarrow N_2O_2$	$\longrightarrow NH_2OH$	$\longrightarrow NH_3$
	+5	+3	+1	−1	−3

Tillman, Sheriha, and Sirny (1965) studied nitrate reduction in sheep and found that the microflora of the rumen required molybdenum to reduce nitrate to nitrite. These results are in agreement with *in vitro* studies, which revealed that bacterial nitrate reductase is a molybdoflavo-protein enzyme (Nicholas, 1959). The Tillman *et al.* study also emphasized that ruminal pH is an important factor in nitrate utilization by rumen microflora. The optimum pH for nitrate reduction is 6.5; for nitrite, 5.6; for hydroxylamine, 6 to 7. These results suggest that

changes in ruminal fluid pH and the presence or absence of metal cofactors are significant factors that determine the rate of appearance of the various intermediates found in the reduction of nitrate to ammonia. Other investigators have attempted to study the relationship of molybdenum excess to nitrate reduction *in vivo*. However, feeding forages high in both molybdenum and nitrate has failed to show any significant effect on ruminal pH, ammonia, or methemoglobin values (Buchman *et al.*, 1966).

Monogastric animals have a relatively high tolerance to nitrates because reduction to the toxic nitrite is minimal. These animals are, however, susceptible to poisoning by preformed nitrites. Reduction of nitrate to nitrite occurs principally in the colon of single-stomached animals. By the time ingesta reaches the colon, most nitrate should have been absorbed from the intestinal tract and excreted in the urine (Whelan, 1935). Equines are reportedly more susceptible to toxicity from nitrate ingestion than other monogastric animals, for the specialized structure of the equine caecum provides conditions similar to those in the rumen — that is, it contains microbial flora capable of reducing the nitrate that reaches this area of the gastrointestinal tract.

The human infant is a significant exception to the general rule that nitrate reduction does not occur in the upper portion of the gastrointestinal tract of monogastric animals. Very young babies have a physiological gastric achlorhydria (absence of free hydrochloric acid in the stomach), which permits nitrate reduction by bacteria in the stomach and duodenum (Burden, 1961; Wolff and Wasserman, 1972).

Variation due to diet. A second factor that may influence nitrite toxicity in livestock is the adequacy of the diet. Inclusion of readily fermentable carbohydrates in the diet is known to protect ruminants against nitrate toxicity (Case, 1957; Wright and Davison, 1964). It has been suggested that carbohydrates increase the rate of disappearance of nitrate in rumen ingesta (Sapiro *et al.*, 1949). Emerick *et al.* (1965) drenched sheep with sucrose or added corn to a forage ration and found that the sheep were protected against orally-administered sodium nitrate but not against sodium nitrite given intravenously. This suggests that variation in severity of nitrate toxicity in sheep is due principally to variation in nitrite accumulation in the digestive tract.

Animals are known to be able to adjust to relatively high levels of nitrate in the feed or water over long periods, although the sudden ingestion of large quantities of nitrate or nitrite will lead to methemoglobinemia (Davison *et al.*, 1962; Sokolowski *et al.*, 1961). Alterations in diet or management may influence the rumen microflora, and changes

in the predominant types of bacteria growing in the rumen can affect the rate at which nitrate and nitrite are reduced.

Little research has been devoted to development of nitrite toxicosis during nutritional deficiencies. One such study was conducted by Kociba and Sleight (1970) to determine the effects of nitrite in ascorbic-acid-deficient guinea pigs. Subcutaneous administration of 50 mg/kg sodium nitrite (10 mg/kg NO_3-N) produced significantly higher levels of methemoglobin and a greater percentage of deaths in the ascorbic-acid-deficient guinea pigs than in the controls, in which no deaths occurred. Moreover, abortions occurred in a high percentage of ascorbic-acid-deficient females following subcutaneous administration of sodium nitrite, but no abortions occurred in nitrite-treated controls or in deficient pregnant females not given nitrite. Although maternal methemoglobin levels were higher in ascorbic-acid-deficient pregnant females following nitrite treatment, methemoglobin levels of fetuses derived by laparohysterotomy were similar in both deficient and control groups. The fetal deaths were apparently related to higher levels of methemoglobin in maternal blood of ascorbic-acid-deficient guinea pigs.

Variation due to metabolic state (age, pregnancy, disease). In man, nitrite has proved more toxic to infants than to adults. Betke, Kleihauer, and Lipps (1956) and Künzer and Schneider (1953) observed that fetal hemoglobin is more easily converted to methemoglobin than is adult hemoglobin. They concluded that as the proportion of fetal hemoglobin to total hemoglobin decreases during infancy, the toxicity risk of nitrite in the infant decreases. Keohane and Metcalf (1960-61) found that sensitivity of erythrocytes to sodium nitrite solutions gradually decreases through infancy and childhood, declining sharply at puberty; under normal conditions, this sensitivity remains at a reduced level throughout adulthood.

Ross and Desforges (1959) and others established that umbilical cord blood erythrocytes were less able to reduce methemoglobin *in vitro* when the level of available DPNH (the reduced form of diphosphopyridine nucleotide) diminished. In comparing 54 samples of cord blood with blood samples from 54 adult controls, Ross (1963) determined that the cord blood was significantly lower in mean level of DPNH-dependent methemoglobin diaphorase (reductase). It was concluded that this transient deficiency of methemoglobin diaphorase might largely account for increased susceptibility of the newborn to acquired methemoglobinemia.

Increased sensitivity of intact erythrocytes has also been demonstrated in pregnant women and in patients with carcinomata (Metcalf,

1960-61). Stress and accelerated division of cells appear to be common predisposing factors in those conditions. Metcalf (1961-62) suggested that the growth process of childhood, the cell replication process in cancer, and the stress of pregnancy remove from the plasma a substance that normally protects the red cells from the effect of nitrite; perhaps this protection involves saving the ferrous iron of the hemoglobin.

Marked differences exist between DPNH-dependent methemoglobin diaphorase activity in the newborn baby and that of the newborn calf (Smith and Beutler, 1966). While the enzyme activity of the human infant is significantly lower than that of an adult, the erythrocyte diaphorase activity of the fetal calf and the newborn calf is spectacularly higher than that of adult cattle. Although bovine fetal hemoglobin is more susceptible to oxidation by nitrite than is adult bovine hemoglobin (Betke, Greinacher, and Tietze, 1956), a higher diaphorase activity may compensate for that susceptibility.

In investigations to compare susceptibility of healthy pigs of various ages to nitrite-induced methemoglobinemia (Emerick *et al.*, 1965), each pig was given sodium nitrite intravenously at the age of one week, again at three months of age, and again at five and one-half months of age. Pigs developed less methemoglobinemia when treated at one week of age than at later intervals. The data appeared to indicate that healthy pigs at one week of age are less susceptible to nitrite-induced methemoglobinemia because they have a higher rate of methemoglobin reduction. Recent studies by Sleight *et al.* (19[illegible]2) indicate that little if any of the fetuses' hemoglobin is converted to methemoglobin during acute episodes of nitrite toxicosis in pregnant sows.

Young rats and pregnant rats have shown increased sensitivity to nitrites. The change in sensitivity does not appear to be correlated to puberty but rather to a relative riboflavine deficiency (Metcalf, 1961-62).

ACUTE TOXICITY

Toxic Doses of Nitrate and Nitrite

In his 1961 review of toxicology of nitrates and nitrites, Burden summarized the available reports in the literature on doses of nitrate and nitrite that produced fatal toxemias. His data are adapted for the summary presented in Table 2.

Since 1960, results from additional experimental studies have extended the knowledge on levels of nitrate and nitrite that will cause acute toxic effects or death. This information, calculated as mg N/kg body weight (except where noted), is summarized as follows:

Table 2. Lethal doses of nitrate and nitrite

	Mg/kg of body weight	
Nitrate	NO_3	N
Adult human	80 to 300	18 to 68
Cattle	330 to 616	75 to 140
Sheep	308	70
Sodium nitrite	NO_2	
Adult human	110 to 165	22 to 33
Rabbits	20 to 90	16 to 18
Pigs	90	18
Sheep	170	34

Lethal doses of nitrate:

Cattle.....169 mg/kg given orally in aqueous solu[illegible] (Hymas and Mesler, 1960)
230 mg/kg LD_{50} consumed with forage (Crawford *et al.*, 1966)

Turkey poults ...600 ppm (LD_{30}) and 1,200 ppm (LD_{90}) in drinking water (Adams *et al.*, 1967)

Nitrate doses producing signs of toxicity:

Cattle (calves)...71[illegible] mg/kg in forage ingested rapidly (Dollahite and Holt, 1970)

Lethal doses of nitrite:

Cattle .....20 mg/kg LD_{50} given as a drench (Wright and Davison, 1964)
45 to 50 mg/kg LD_{50} given to simulate eating (Wright and Davison, 1964)

Sheep .....90 mg/kg given intraperitoneally (Diven *et al.*, 1964)
64 mg/kg given rapidly intraruminally (Sinclair and Jones, 1967)

Swine .....12 to 20 mg/kg given orally (Michalski, 1963)
8 to 12 mg/kg given subcutaneously (Michalski, 1963)
21.35 mg/kg LD_{100} given via stomach tube (Curtin and London, 1966)
15 to 18 mg/kg LD_{50} given via stomach tube (Wright and Davison, 1964)

Rats.....15 to 18 mg/kg LD_{50} given via stomach tube (Wright and Davison, 1964)

Dogs.....12 to 30 mg/kg given orally (Michalski, 1963)
8 to 12 mg/kg given subcutaneously (Michalski, 1963)

Nitrite doses producing signs of toxicity:

Sheep.....24.5 mg/kg given intravenously (Bartík, 1967)

Swine.....12.2 to 19.3 mg/kg given via stomach tube (Curtin and London, 1966)

Chicks or turkey poults....200 ppm in drinking water (Adams *et al.*, 1966)

Mice.....31.6 mg/kg (2.29 mM/kg $NaNO_2$) LD_{50} given intraperitoneally (Smith and Layne, 1969)

Signs and Lesions

The signs of acute nitrite toxicity in all animals are primarily those of hypoxia; death occurs from anoxia due to methemoglobinemia.

In ruminants the first noticeable sign usually is a brownish discoloration of nonpigmented areas of the skin and mucous membranes resulting from the chocolate-brown color of methemoglobin in the blood. Staggering gait, rapid pulse, frequent urination, diarrhea, and labored breathing appear, followed by collapse. In lethal intoxications, convulsions and coma may ensue, culminating in death within 1 to 3 hours after onset of the signs. An animal that has received a sublethal dose of nitrite may recover following collapse. Pregnant cows that have survived a nearly fatal methemoglobinemia may abort within a few days (Wright and Davison, 1964). The threshold for the lethal effect of nitrite varies, but death usually occurs after 70 to 80 percent of hemoglobin has been converted to methemoglobin (Dodd, 1966; Curtin and London, 1966).

Affected monogastric animals often suffer vomiting and diarrhea, apparently from irritation of the intestinal mucosa by high concentrations of nitrite. Signs in experimentally poisoned swine are similar to those in ruminants (Curtin and London, 1966). Acute nitrite toxicity is manifest in apparently healthy human infants by sudden onset of cyanosis, often accompanied by listlessness and anorexia and occasionally dyspnea.

Post mortem findings in animals that have died from nitrite poisoning are indicative of methemoglobinemia and respiratory distress — that is, chocolate-brown discoloration of the blood and tissues, cardiac

hemorrhages, and pulmonary congestion (Dodd, 1966; Wright and Davison, 1964).

An attempt to study the nitrite-induced anoxic state at the molecular level was reported by Uzoukwu and Sleight (1970). Nitrite toxicosis was produced in three groups of guinea pigs by administration of sodium nitrite (1) in drinking water, (2) by subcutaneous injection, or (3) by confinement in a mixture of air and nitrogen. In all groups the activity of lactic dehydrogenase and succinic dehydrogenase (two enzymes essential for reduction of methemoglobin to hemoglobin in the erythrocyte) in the myocardium was appreciably reduced, and in the latter two groups the activity of both enzymes in the kidney was localized. These alterations in enzyme activity probably accounted for subsequent histologic changes: hyperemia of liver, spleen, kidneys, and lungs and vacuolar degeneration of hepatic cells and renal tubular epithelium. The vasodilatory effect of nitrite probably contributed to the hyperemia observed.

Diagnosis

The diagnosis of acute nitrite toxicity must be based on a history of ingestion of feed or water that may contain excessive levels of nitrate or nitrite; nitrate/nitrite determinations of the suspected feed or water; signs of hypoxia; nitrite or methemoglobin determinations of the blood; and typical post mortem findings. The most useful indication of poisoning is the brownish discoloration of the tissues and blood. A comparison of clinical effects of common toxicants that may be confused with nitrate/nitrites is given below (adapted from Buck, 1970).

Toxic factor	Blood color	Mechanism	Treatment
Nitrate/nitrite	Brown	Methemoglobinemia	Methylene blue
Sodium chlorate (a herbicide)	Brown	Methemoglobinemia	Methylene blue
Silo gases	Slightly brown	Slight methemoglobinemia	Methylene blue; Ca gluconate
Cyanide	Cherry red	Anti-cytochrome: tissues cannot utilize oxygen	Nitrite thiosulfate
Carbon dioxide	Dark blue	Displaces oxygen; cyanosis	Oxygen; fresh air
Carbon monoxide	Bright red	Carbon monoxide hemoglobin complex (stable)	Oxygen + 5 percent thionine solution; fresh air

Since blood hemoglobin is rapidly converted to methemoglobin, blood to be examined for methemoglobin level must be drawn while the animal is alive, or within 2 hours after death, and must be analyzed within an hour. For examination by spectrophotometry, an anticoagulant must be added to the blood (Dodd, 1966). The methemoglobin content of human blood taken from persons having died from nitrite has been observed to diminish after death unless samples are refrigerated at 4° C (Werner *et al.*, 1965). If the blood cannot be analyzed immediately, it may be kept for 24 hours if it is oxalated and cooled to 5° C or is hemolyzed by dissolving 1 ml blood in 20 to 50 ml distilled water (Bajo *et al.*, 1965). Dilution of the blood in 20 volumes of phosphate buffer (pH 6.6) has been reported to maintain methemoglobin stability for 48 hours (Watts *et al.*, 1969).

Nitrate/Nitrite Determinations

Nitrate/nitrite determinations should be performed on body fluids (serum; plasma; fetal bile; urine; thoracic, pericardial, or peritoneal fluid) of poisoned animals to confirm the diagnosis. The laboratory determination of nitrate/nitrite is based on spectrophotometric analysis of a diazotization coupling: in the presence of nitrite, sulfanilic acid reacts with 1-naphthylamine to form a red dye (Nelson *et al.*, 1954). Details of methods and variations used in nitrate/nitrite determinations are given in the review by Hanway *et al.* (1963). Additional techniques have been recommended for analyzing blood nitrate/nitrite levels (Diven, Pistor, *et al.*, 1962; Marich and Asbury, 1965; Litchfield, 1967) and levels in foods, feeds, and soil (Woolley *et al.*, 1960; Manning *et al.*, 1968; Adriaanse and Robbers, 1969; Mehnert, 1968).

The improved xylenol nitration method for body-fluid nitrate analysis is a useful diagnostic test. Since the nitro-complex formed is more stable than a diazo-complex, a veterinarian has ample time to send samples to the laboratory and have reasonable confidence in the results (Greweling *et al.*, 1964; Kühnert, 1967).

A technique involving chromatographic separation of extracts from silages or forages has improved nitrate analysis by removing interfering substances (Wiseman and Jacobson, 1965). Analysis of water for nitrates has been performed efficiently by using an Orion specific-ion meter. This method has been recommended for routine analysis of well and surface waters in which nitrate levels approach or exceed the tolerance limit (Keeney *et al.*, 1970).

A simple and accurate field test that detects nitrate or nitrite in samples from experimentally poisoned animals up to 24 hours after

death has been recommended by Housholder *et al.* (1966). Three drops of a diphenylamine test reagent are mixed with a drop of suspect fluid on a white spot plate. An immediate intense blue color indicates nitrate/nitrite reaction. Since false positive results can be obtained with bromides, chlorates, selenites, molybdates, iron, antimony, and peroxides, signs of the poisoning must be evaluated along with the test.

Treatment

Animals suffering from nitrite- or hydroxylamine-induced methemoglobinemia should be treated immediately with intravenously administered methylene blue solution. Methylene blue is thought to act as an electron acceptor for the methemoglobin reductase enzyme in the blood, thus speeding the reconversion of methemoglobin to hemoglobin. Bradley *et al.* (1940) injected a 1- to 4-percent aqueous solution at the rate of 2 grams of methylene blue per 500 pounds of body weight. The methylene blue may be used in a 5 percent glucose solution or a 1.8 percent sodium sulfate solution (Case, 1957). Humans are usually treated by a slow intravenous injection of a 1-percent aqueous solution of methylene blue in a dosage not exceeding 1 mg/kg body weight (Schaffer, 1960). Experimental studies of therapy for methemoglobinemia in swine showed that 2 to 8 mg/kg of methylene blue administered intravenously gave adequate reduction to hemoglobin. Sympathomim (sympathol, 4-oxyphenyl-β-methylaminoethanol) at 5mg/kg body weight was one of the most effective and safest of several drugs evaluated to raise the blood pressure of experimentally poisoned pigs (Kovács *et al.*, 1965). Experimentally, solutions of toluidine blue and thionine have also decreased methemoglobin levels with no adverse side reactions. In the same study, vitamin C (100 mg/kg) given intravenously and vitamin A (12,500 units/kg) given intramuscularly had no effect on reducing methemoglobin (Tai-hsiumg and Yu, 1965).

CHRONIC OR SUBLETHAL TOXICITY

Interest in chronic or sublethal effects of nitrates was stimulated by events in Missouri after a severe drought in 1954. Poorly nourished livestock consumed forages containing high levels of nitrate; subsequently, some developed methemoglobinemia and died of acute nitrate intoxication. Losses in meat and milk production occurred in other animals that had consumed excessive but sublethal levels of nitrates (Brady *et al.*, 1955; Case, 1957). Numerous field reports and experimental studies subsequently incriminated nitrate as a causal or contributing factor in a variety of conditions, including reduced growth

and production, abortion, and interference with iodine and vitamin A metabolism. More recent attempts to substantiate early reports on sublethal effects of nitrate consumption have produced varied and often contradictory results.

Nitrates and the Thyroid Gland

Most early research on the relationship of nitrate consumption to thyroid function was done with rats (see review by Wright and Davison, 1964). A general conclusion that may be drawn from these studies is that elevated levels of dietary nitrate interfere with normal thyroid function (Bloomfield *et al.*, 1961; Bloomfield, Welsch, *et al.*, 1962; Welsch *et al.*, 1961; Wyngaarden *et al.*, 1952). Wyngaarden *et al.* found that nitrate and nitrite, like other monovalent anions, compete with iodine in the thyroid of rats and thus interfere with thyroxine synthesis. This finding was confirmed by Bloomfield *et al.* (1961), who suggested that the dietary level of iodine is an important factor when nitrate is present in the diet. It was subsequently found by Bloomfield, Welsch, *et al.* (1962) that decreased thyroidal I^{131} uptake occurs in rats maintained on a nitrate-supplemented diet. However, the effect was overcome after two weeks as a result of thyroidal compensation.

An investigation to determine the effect of dietary nitrate on anterior pituitary and thyroid gland function in cattle was conducted by Jainudeen *et al.* (1965). Feed of dairy heifers was supplemented with nitrate at a level of 0, 440, or 660 mg nitrate/kg body weight (0, 100, or 150 mg/kg NO_3-N). Pituitaries from nitrate-fed heifers were significantly heavier than those from control animals and contained a slightly higher concentration of growth hormone. However, nitrate did not affect thyrotropic, adrenocorticotropic, or gonadotropic activity of pituitary glands. Levels of nitrate used in the study failed to produce histologic change in thyroid glands or any evidence of thyroid dysfunction.

The effect of dietary nitrate on thyroxine secretion in lambs adapted to nitrate-supplemented diets and in lambs not adapted to nitrate-supplemented diets was studied by Arora *et al.* (1968). One group of lambs received a basal diet; a second group received the basal diet for 50 days, after which the diet was supplemented with 4 percent potassium nitrate (0.56 percent NO_3-N). A third group received the nitrate-supplemented diet throughout the study. Lambs in each group were given I^{131} intramuscularly on the fiftieth day of the experiment, and thyroxine secretion rates were then determined. Daily thyroxine secretion in the nonadapted (second) group was lower, but not significantly

so, than in the control group. The nitrate-adapted (third) group secreted the same level of thyroxine as the controls. These results, coupled with analysis of growth rates of lambs, indicate that sheep may adapt to relatively high levels of nitrates in the diet so as to circumvent thyroidal dysfunction.

It may be concluded that if dietary nitrates interfere with thyroid function, the effect is a transitory one not likely to lead to hypothyroidism and its sequelae.

Nitrates and Thyroid-Vitamin A Relationships

Johnson and Baumann (1947) demonstrated that an active thyroid gland in rats increased intestinal absorption of carotene and conversion of carotene to vitamin A. It was later suggested by Bloomfield *et al.* (1961) that vitamin A deficiencies, observed in cattle that consumed nitrates, might be an indirect result of abnormal thyroid function induced by the nitrate. Evidence obtained more recently argues against this view.

Effects of dietary potassium nitrate and treatment with tapazole (a thyroid inhibitor), vitamin A, and vitamin E on weight gains and liver storage of vitamin A have been studied in fattening lambs (Cline *et al.*, 1963). A basal ration supplemented with 4-percent potassium nitrate (0.56-percent NO_3-N) did not reduce weight gain or affect the vitamin A status of lambs when fed separately or in combination with weekly injections of tapazole. Jordan *et al.* (1963), in studies designed to induce vitamin A deficiency in feeder steers, concluded that high environmental temperature and high nitrates in feed may jointly induce hypothyroidism with resulting vitamin A deficiency.

Continuous feeding of sublethal levels of nitrate to dairy heifers has failed to produce thyroid dysfunction and vitamin A deficiency (Jainudeen *et al.*, 1965; Wright and Davison, 1964). According to Wright and Davison, "... it appears that a ruminant would probably die of toxicity before the level of nitrate or nitrite in any part of the body could reach a concentration great enough to interfere with iodine or vitamin A nutrition."

Effect of Nitrates on Vitamin A Metabolism

Nitrates or nitrites in feed or water may lead to vitamin A deficiencies in livestock by destroying carotene or by interfering with the utilization of vitamin A (National Academy of Sciences, 1972).

It was reported in 1965 that nitrate or nitrite in the diet of rats increased carotene disappearance from the small intestine (Mitchell *et al.*,

1965). The level of carotene found in serum was also increased, thus leading the authors to suggest that nitrate may stimulate absorption of intact carotene before it can be converted to vitamin A. Phillips (1966) demonstrated that livers of rats fed 1-percent potassium nitrite (0.16-percent NO_2-N) in diets supplemented with carotene or vitamin A contained less vitamin A than those of rats given no nitrite. It was concluded that nitrite degrades carotene and vitamin A before they can be absorbed in the digestive tract.

Several other reports indicate that nitrite consumption inhibits accumulation of liver vitamin A in laboratory rodents (Emerick and Olson, 1962; O'Dell *et al.*, 1960; Yadav *et al.*, 1962), and in poultry (Adams *et al.*, 1966; Bentley *et al.*, 1965; Marrett and Sunde, 1968; Sell *et al.*, 1963; Sell and Roberts, 1963; Tomov, 1965).

Studies designed to determine the effects of nitrate or nitrite on vitamin A metabolism in swine have produced variable results. Seerley *et al.* (1965) added sodium nitrate and sodium nitrite to drinking water to provide up to 300 ppm NO_3-N and 100 ppm NO_2-N, respectively, to swine but were unable to detect changes in liver vitamin A after 125 days of treatment. Conversely, swine receiving 0.3-percent nitrate (690 ppm NO_3-N) in drinking water were shown to have reduced liver vitamin A if they received supplemental beta carotene but not if they received preformed vitamin A (Wood *et al.*, 1967). Water containing 0.08-percent nitrite (240 ppm NO_2-N) reduced vitamin A stores in swine regardless of the form of supplemental vitamin A fed.

In a continuation of the latter investigation, Hutagalung *et al.* (1968) added potassium nitrate and potassium nitrite to the diets of pigs to study their effect on the utilization of dietary carotene. Although ingestion of a ration containing 3-percent nitrate (0.69-percent NO_3-N) supplemented with beta carotene had no adverse effect on liver vitamin A stores, adding 0.3-percent nitrite (0.09-percent NO_2-N) to the rations produced a definite but insignificant trend toward decrease in liver vitamin A stores. These results appear to agree with those reported by others (Koch *et al.*, 1963; London *et al.*, 1967).

The preponderance of existing evidence indicates that, in ruminants, dietary nitrates do not interfere with liver storage of vitamin A (Cline *et al.*, 1963; Davison, Hansel, *et al.*, 1964; Davison, McEntee, *et al.*, 1964; Hale *et al.*, 1961; Jones *et al.*, 1966; O'Donovan and Conway, 1968; Smith *et al.*, 1962; Sokolowski *et al.*, 1961; Wallace *et al.*, 1964; Weichenthal *et al.*, 1963). Dietary nitrate seems to exert its greatest influence on vitamin A by reducing the amount of dietary vitamin A that reaches hepatic stores rather than accelerating the depletion of existing

stores (Hoar *et al.*, 1968). Indeed, some investigators have shown that the more toxic reduction products of nitrate (nitrite and hydroxylamine) have no significant effect on vitamin A status in sheep and cattle even though the levels used were sufficient to raise methemoglobin levels in the blood (Tillman, Sheriha, Goodrich, *et al.*, 1965; Cunningham *et al.*, 1968).

In vitro destruction of vitamin A has been observed in rumen and abomasal fluids (Klatte *et al.*, 1964). Slightly more vitamin A was destroyed when a known amount was incubated anaerobically at 37° C for 4 hours in ruminal fluids than when treated similarly with abomasal fluids; in both cases, losses were more than twice the loss from incubations of vitamin A in autoclaved ruminal fluids. These results suggest that microflora of the rumen may play an active role in destruction of vitamin A.

Studies with nitrite solutions have provided evidence that carotene and vitamin A are destroyed when incubated with nitrites at low pH (Pugh and Garner, 1963). Olson *et al.* (1963) also demonstrated that high levels of nitrite, but not nitrate, caused rapid destruction of carotene in an acid medium but not at neutral or alkaline pH.

Results obtained from *in vitro* incubation of beta carotene with rumen fluid from nitrate-fed or control heifers indicated that nitrate did not increase carotene destruction in the rumen (Davison and Seo, 1963). These results are similar to those of Olson *et al.* (1963), who found, however, that addition of nitrite to abomasal fluid caused almost complete destruction of carotene. In another such study, steers and wethers were fed high-roughage rations that contained approximately 0.14-percent NO_3-N. Samples taken from abomasal fistulas showed that dietary nitrate had not altered preintestinal destruction of vitamin A (Mitchell *et al.*, 1967). This result was confirmed by Cunningham *et al.* (1968), who found that vitamin A and carotene destruction in rumen fluids incubated *in vitro* was no more rapid in fluids from nitrate- and nitrite-fed steers than in those from control steers.

Roberts and Sell (1963) investigated the destruction of vitamin A by nitrite in sheep and chickens. In crop and intestinal fluids having a pH above 6 and in rumen fluids, relatively little vitamin A was destroyed in 60 minutes of *in vitro* incubation with 2-percent potassium nitrite solution (0.32-percent NO_2-N). In the ventricular fluid, with a pH below 4, vitamin A destruction was rapid. When this level of nitrite was fed to fistulated sheep, no nitrite was found in either the rumen or abomasal fluid 90 minutes after feeding, and there was relatively little difference in vitamin A concentration between nitrite-fed sheep and controls. Since nitrite was not detected in the abomasal fluid, the nitrite

was either metabolized or absorbed before reaching the abomasum. Broilers fed 0.74-percent potassium nitrite (0.12-percent NO_2-N) had nitrite in the crop, ventriculus, and intestine 2 hours after feeding and, compared with controls, had a reduced vitamin A content in the ingesta.

Nitrate/nitrite in the feed has been implicated in the avitaminosis A seen in cattle fed silage supplemented with vitamin A (Smith *et al.*, 1961; Jordan *et al.*, 1961). Data published and reviewed by Emerick *et al.* (1963) suggest that perhaps carotene and vitamin A losses occur in silage before they ever reach the stomach of an animal. During silage fermentation, nitrates are converted to nitrite and other reduction products. Since silage fermentation provides an acid medium, which is the proper pH for carotene destruction by nitrite, increased nitrates in plants used for silages may increase carotene losses, especially during the first week of storage. Emerick concluded, "... although other mechanisms may be involved, the most important effect of nitrate on the vitamin A status of livestock is probably a result of the direct destruction of carotene in certain feeds prior to their consumption by the animal."

It appears that the nitrate/nitrite effect on vitamin A nutrition in animals may be summarized as follows. Nitrite, but not nitrate, destroys carotene or vitamin A at a pH below 4. Formation of nitrite from nitrate requires a pH near neutrality, such as that which occurs in the rumen; once formed, however, nitrite normally is absorbed or reduced so rapidly that little if any would be found in the acid abomasum. Thus, nitrite destruction of vitamin A in the ruminant digestive tract appears unlikely. Under conditions in which nitrite accumulates in the digestive tract, such as dietary molybdenum excess or a copper or iron deficiency that limits nitrite reduction, nitrite could be responsible for vitamin A destruction, but acute nitrite toxicity would be of more vital concern in such a case.

In monogastric animals, the normal adult animal does not reduce nitrate to nitrite in significant amounts because acid conditions of the stomach inhibit nitrate/nitrite-reducing bacteria. However, preformed nitrite is not likely to be so rapidly reduced or absorbed as in ruminants. Carotene and vitamin A may be destroyed by nitrite in this acid environment, even under long-term consumption of sublethal doses.

Effects on Production

Nitrates in the ration or in drinking water have been associated with reduced weight gain and reduced milk or egg production. Recent

studies and retrospective examinations of early publications reveal that decreases in production appear to coincide with reduced consumption of feed or water when their levels of nitrate content are sufficient to reduce palatability. This was true for swine (Hutagalung *et al.*, 1968; Koch *et al.*, 1963; Tollett *et al.*, 1960; Wood *et al.*, 1967), chicks (Sell and Roberts, 1963; Sell *et al.*, 1963), and cattle (Cunningham *et al.*, 1968; Hale *et al.*, 1962; Jones *et al.*, 1966).

Other experimental studies have revealed no effect of long-term, low-level nitrate consumption on gain, feed conversion, or production in swine (London *et al.*, 1967), sheep and swine (Seerley *et al.*, 1965), sheep (Cline *et al.*, 1962; 1963), cattle (Crawford and Kennedy, 1960; Crawford *et al.*, 1966; Davison *et al.*, 1962; Davison, Hansel, *et al.*, 1964; Jones *et al.*, 1966; Smith *et al.*, 1962; Wallace *et al.*, 1964), and turkey poults (Adams *et al.*, 1967; 1969; Kienholz *et al.*, 1965).

Growth reductions and poor production have been seen in poultry in conjunction with vitamin A deficiencies induced by nitrite in the feed or in drinking water (Adams *et al.*, 1966; Bentley *et al.*, 1965; Marrett and Sunde, 1968; Sell and Roberts, 1963; Tomov, 1965). Nitrate at 2.5-percent sodium nitrate (0.4-percent NO_3-N) significantly reduced weight gains in rapidly growing lambs that were being depleted in vitamin A or receiving modest supplements of vitamin A or carotene (Hoar *et al.*, 1968). Drinking water containing 0.08-percent nitrite (240 ppm NO_3-N) significantly reduced feed consumption and weight gains in swine but also reduced liver vitamin A stores (Wood *et al.*, 1967).

It is difficult to interpret two reports of increased weight gain in chicks and turkey poults receiving nitrate in drinking water. Birds that received 675 ppm nitrate (153 ppm NO_3-N) until four weeks of age were significantly heavier than controls when both groups were 16 weeks of age. Growth response did not increase when higher nitrate levels were added to the water. Nitrate-treated birds had considerably smaller testes. Thyroid and adrenal weights did not differ from those of controls. A hormone imbalance that increased energy retention was postulated to explain these results (Arends *et al.*, 1967; Kienholz *et al.*, 1965).

Abortions

Early descriptions of nitrate/nitrite-induced methemoglobinemia refer to abortion in cows as a sequel to acute nitrite intoxication (Bradley *et al.*, 1940). Subsequent reports claimed that ingestion of sublethal amounts of nitrate caused abortion in livestock. Missouri workers (Garner, 1958; Muhrer *et al.*, 1956) suspected that abortions in apparently

disease-free cattle occurred after the cattle had consumed forages containing about 1 percent potassium nitrate (0.14 percent NO_3-N). Nonspecific abortions in Wisconsin cattle grazing on weeds were thought to be due to accumulations of nitrates in these plants (Simon *et al.*, 1958; Simon, Sund, Wright, and Douglas, 1959). Wisconsin investigators were unable to reproduce the syndrome with low doses of nitrate or nitrite, but 100 g of potassium nitrate (14 g NO_3-N) given each day via rumen fistula caused abortions in three heifers (Simon, Sund, Douglas, *et al.*, 1959).

In a well-controlled study by Davison, Hansel, *et al.* (1964), 45 heifers were fed 0, 100, or 150 mg NO_3-N/kg body weight daily, beginning three estrous cycles before breeding or at 40, 150, or 240 days of pregnancy. Two heifers in the group with the highest daily dose died. Three heifers aborted: one had been fed at the lower nitrate level, the other two were in the group fed the higher level. Estrous cycles, gestation period, placentas, and reproductive tracts of the remaining heifers and birth weights and performance of offspring were all within normal limits.

Winter and Hokanson (1964) were unable to relate abortions to long-term feeding of nitrate and nitrite. Heifers were fed a balanced daily ration to which was added sufficient sodium nitrate or sodium nitrite to maintain methemoglobin levels at 20 to 30 percent or 40 to 50 percent. The ration was fed from the time the heifers were two months pregnant until pregnancy was terminated. A causal relationship could not be shown between the treatments and the three abortions that occurred, nor were significant lesions observed on gross pathologic examination. The authors suggested that heifers given an adequate ration can tolerate for prolonged periods the ingestion of quantities of nitrate and nitrite sufficient to convert 40 to 50 percent or more of total hemoglobin to methemoglobin with no detrimental effects on the maintenance of pregnancy.

Corn silage supplemented with 0.75-percent potassium nitrate (0.11-percent NO_3-N) failed to noticeably affect reproduction performance when fed to dairy cows for nine weeks during mid-lactation pregnancy (Jones *et al.*, 1966). Oat hay containing up to 2.3 percent nitrate (0.52 percent NO_3-N) did not cause abortions when fed to pregnant cows for 25 to 35 days (Crawford *et al.*, 1966).

Oat hay containing 0.8-percent potassium nitrate (0.11-percent NO_3-N) produced no harmful effects when fed to ewes throughout pregnancy (Eppson *et al.*, 1960). Sinclair and Jones (1964) failed to produce abortions in sheep fed a ration containing approximately 1.5

percent potassium nitrate (0.21 percent NO_3-N) during the last three to four months of pregnancy. Another study reported results when pregnant ewes were fed forages supplemented with nitrate (Davison *et al.*, 1965). Forages containing 1.5 percent nitrate (0.34 percent NO_3-N) did not produce significant reproductive problems; abortions occurred, however, in three of seven ewes fed forage containing 3.4 percent nitrate (0.77 percent NO_3-N).

Long-term consumption of nitrates seems to have little effect on reproduction in swine. Seerley *et al.* (1965) found that up to 300 ppm NO_3-N and 100 ppm NO_2-N did not affect thriftiness, breeding, or reproduction of gilts through two farrowings. This confirmed an earlier report by Tollett *et al.* (1960).

Reproduction has been more thoroughly investigated experimentally in the guinea pig. Potassium nitrate in doses ranging from 300 to 30,000 ppm (42 to 4,200 ppm NO_3-N) and potassium nitrite from 300 to 10,000 ppm (48 to 1,600 ppm NO_2-N) were given in the drinking water for periods of 100 to 240 days. Reproductive performance in females given 30,000 ppm potassium nitrate (4,200 ppm NO_3-N) was only 8 percent of that of controls; in females given 5,000 and 10,000 ppm potassium nitrite (800 and 1,600 ppm NO_2-N) fetal losses were 100 percent. At lower levels of treatment, reproduction was maintained. In females that had aborted or had mummified fetuses, uterine and cervical inflammatory lesions and placental degeneration were observed. Hypoxia due to brief, severe maternal methemoglobinemia was suggested as the cause of death of fetuses as well as maternal losses. Unfortunately, records of methemoglobin levels were incomplete. Male fertility apparently was not impaired, as conception took place at all levels. Normal reproduction at 3,000 ppm nitrite (480 ppm NO_2-N) and 10,000 ppm nitrate (1,390 ppm NO_3-N) indicates considerable tolerance to toxicity in these laboratory animals (Sleight and Atallah, 1968).

Erythropoietic Response

One manifestation of long-term consumption of sublethal levels of nitrate/nitrite appears to be a compensatory erythropoietic response to abnormal levels of methemoglobin in the blood. Jainudeen *et al.* (1964) fed daily 440 to 660 mg nitrate (100 to 150 mg NO_3-N) per kg body weight to pregnant heifers for prolonged periods. When compared with controls, nitrate-fed animals demonstrated marked increases in hemoglobin concentrations, packed cell volumes, and blood volumes. Increases were not attributed to diuresis or dehydration, since plasma-

volume levels were similar to levels in control animals. Similar results have been noted by others in cattle (Davison *et al.*, 1962; Winter and Hokanson, 1964), swine (Curtin and London, 1966; London *et al.*, 1967), and turkeys (Adams *et al.*, 1967). It appears that the hypoxia induced by gradual conversion of hemoglobin to methemoglobin, and the resultant erythropoietic response, may partly explain why animals are able to adapt to relatively high and long-term levels of nitrate in feed or water.

TOXICITY OF NITROGEN OXIDES

Oxides of nitrogen, which may be produced in a variety of industrial and agricultural processes, represent a potential hazard to livestock and human health. When oxides of nitrogen are inhaled, they combine with the aqueous film lining the respiratory passages to produce nitrous and nitric acids. High concentrations of the gases result in damage to alveolar capillaries; pulmonary edema, hemorrhage, and fibrin formation follow (Giddens *et al.*, 1970). The term "silo-filler's disease" has been used to describe nitrogen dioxide toxicity in persons who have inhaled gases produced in silos recently filled with forage.

When forages are ensiled, enzymes from bacteria or from the plants themselves may anaerobically reduce nitrate to nitrite; eventually, the following series of reactions may occur:

	reduction	$+O_2$	$+O_2$	
Nitrite	Nitric oxide	Nitrogen dioxide		Nitrogen tetroxide
NO_2^-	NO (colorless gas)	NO_2 (brown gas)		N_2O_4 (yellow gas)

According to Weisburd (1962), summarizing the toxicity of nitrogen dioxide gas to humans, a concentration of 700 or more ppm of the gas is fatal in 30 minutes or less; exposure to 100 to 150 ppm for 30 minutes to an hour is dangerous; more than 62 ppm will cause immediate throat irritation; 5 ppm is the odor threshold and the maximum allowable concentration for 8-hour exposure. Moreover, when sulfur dioxide is also present, nitrogen dioxide toxicity is increased, with the result that as little as 2 ppm nitrogen dioxide may affect animals already suffering from any form of chronic lung insufficiency (Shalamberidze, 1969).

A similarity of lesions exists among "silo-filler's disease" in man, pulmonary adenomatosis in cattle (Seaton, 1957), and experimental

cases of nitrogen dioxide poisoning in laboratory animals (Haydon *et al.*, 1967; Kleinerman and Wright, 1961; Riddick *et al.*, 1968). Rats, rabbits, guinea pigs, and dogs tend to demonstrate a similar physiological and histopathological picture when continuously exposed to low levels of nitrogen dioxide. Early reversible pulmonary edema is followed by nonreversible emphysema and peribronchial/perialveolar increases in collagen and reticulum fibers.

In a more recent study, pigs experimentally exposed to high concentrations of silo gas for 10 minutes or more died in 21 to 72 minutes (Giddens *et al.*, 1970). Analysis of the gas revealed a content of up to 140 ppm nitric oxide and up to 360 ppm nitrogen dioxide. Early lesions in pigs that died from the inhaled gas were pulmonary alveolar edema and hyperemia. Pigs that survived the longest developed hemorrhage; bronchiolar and bronchial epithelial desquamation; and perivascular, interlobular, and subpleural edema. However, bronchiolar epithelial necrosis, an essential feature in development of "silo-filler's disease" in man, did not occur in pigs that survived the acute effects of silo gas toxicosis.

NITROSAMINES

With the discovery that nitrosamines may be toxigenic and even carcinogenic, another dimension has been added to the "chronic" effects of nitrite. Magee (1973) recently estimated that more than 100 nitrosamines are capable of producing tumors in laboratory animals.

Nitrosamines are formed by the interaction between nitrites and secondary or tertiary amines under conditions similar to those found in the mammalian stomach (Lijinsky and Epstein, 1970). The following scheme, based on discussion by Sander (1969-70) and by Sander and Seif (1969), may be postulated for formation of nitrosamine in the human stomach; the first step has been demonstrated to occur in the human stomach if an antacid drink is able to maintain the pH above 4 for three hours.

Nitrate		Nitrite		Nitrosyl ion		Secondary amine		Nitrosamines
NO_3^-	$\longrightarrow$	NO_2^-	$\longrightarrow$	NO^+	+	R-N-R′	$\longrightarrow$	R-N-R′
						|		|
						H		N
								||
								O
(bacterial reduction at pH 4 to 7 before or after ingestion)		(at pH 1 to 3; values of human stomach)				(formed from protein foods — especially during cooking)		

There have been numerous reports of the induction of tumors in experimental animals by nitrosamines (Druckrey *et al.*, 1963; 1967; Magee and Barnes, 1967; Sander, 1970; Sander and Bürkle, 1969; Sen *et al.*, 1969; Terracini *et al.*, 1967). While nitroso compounds are known to induce tumors in most organs of the rat, results of feeding studies are not yet decisive as to precise dose-response relationships (Wolff and Wasserman, 1972). There is increasing evidence that nitroso compounds of different structure may induce tumors in different organs (Magee, 1973). Some nitrosamines may induce tumors after only one dose; some have induced tumors in the progeny of pregnant rats; others are powerful mutagens. At least one investigation (Sen *et al.*, 1969) suggests that rabbits and cats would be more suitable for experiments in which are sought data for extrapolation to man, since the gastric juice pH of these animals is similar to that of man.

The hazard to livestock populations from nitroso compounds appears to be limited to unusual circumstances that may result in ingestion of relatively high levels of a toxic nitrosamine. Such circumstances existed in Norway in 1961 and 1962, when herring meal containing high levels of dimethylnitrosamine (DMN) was found to be responsible for extensive centrilobular liver necrosis, fibrosis, and death of cattle, sheep, and chicks (Koppang, 1964; Koppang and Slagsvold, 1964). It was concluded that the DMN was formed during processing of the herring meal when large concentrations of sodium nitrite were added. Analysis of the meal revealed that some batches contained 30 to 100 ppm DMN; these batches produced toxic hepatosis when fed experimentally to sheep (Sakshaug *et al.*, 1965).

Similar cases of liver disease occurred on mink and fox farms in Norway; these, too, were found to be related to ingestion of DMN-laden herring meal (Koppang, 1966). Toxic hepatosis like that in Norway is not likely to become a problem for the U.S. livestock industry unless use of nitrite-preserved feeds such as fish meals becomes a more common practice here.

Nitrates and nitrites are commonly used to preserve meat, fish, and cheese for human consumption. Nitrite, either purposely added or formed from nitrate by bacteria in the meat, may react with myoglobin or hemoglobin to form nitrosomyoglobin and nitrosohemoglobin. These pigments impart a red color to the meat. *In vitro* tests have shown that nitrosamines can be formed in the acidic human gastric juice from secondary amines and nitrites (Sander, 1967).

An interesting case of nitrite-associated cancer has been described by DuPlessis *et al.* (1969). A high incidence of esophageal cancer

in Bantu people (mainly the women) in localized areas of Transkei was found to be associated with a molybdenum deficiency in the leaves of their food plants. The lack of molybdenum prevented nitrogen metabolism in the plants and led to abnormal nitrate accumulations. A search for possible nitrosamines in the Bantu foods led to the discovery that DMN was present in *Solanum incanum* fruit, the juice of which was used to curdle milk. These curds were the chief source of sustenance for women and boys.

There is no evidence that foods consumed by persons in the United States contain sufficient levels of nitrosamines to induce carcinogenic, mutagenic, or teratogenic effects (National Academy of Sciences, 1972).

RECENT FIELD CASES OF NITRITE TOXICITY IN LIVESTOCK

The few well-documented field cases of nitrite toxicity described in the literature of the past decade emphasize the importance of exhaustive epidemiologic studies to identify complicating factors that may be essential to development of nitrite toxicity in livestock.

Nitrite poisoning of cattle occurred in the western district of Victoria, Australia (Harris and Rhodes, 1969), when cattle under severe nutritional stress were permitted sudden access to drought-depleted pastures that contained succulent growths of *Arctotheca calendula* (capeweed). In an area of about 40 km radius, approximately 400 cattle were affected, of which 130 died. Of 180 acutely affected animals treated with methylene blue, all but one recovered. Post mortem lesions were typical for methemoglobinemia, and analysis of capeweed revealed dry-matter nitrate levels between 2.0 and 4.4 percent (0.45 to 0.99 percent NO_3-N).

Feed-induced nitrite poisoning in swine was reported from Hungary (Kozma and Szilágyi, 1967), where deaths of pigs from methemoglobinemia occurred in several isolated villages. Large amounts of *Xanthium strummarium* seedlings were found on post mortem in stomach contents of affected pigs. These seedlings contained 4 to 5 g/kg of potassium nitrate (560 to 700 mg/kg NO_3-N). Analysis of well water revealed that the swine had also been consuming water in which nitrate levels varied from 517 to 1,360 ppm (119 to 313 ppm NO_3-N). These findings led the authors to suggest that high levels of nitrate in drinking water might have been responsible for sporadic cases of nitrite intoxication, while additional nitrate intake from *Xanthium* seedlings precipitated acute nitrite toxicity.

Andersen (1962) reported an interesting case of methemoglobinemia in pigs that occurred after they had consumed condensate water from ventilating shafts; this water contained 2,310 to 3,300 ppm

NO_2-N. In a later study of ventilation systems in 23 pighouses, Hovmand and Slot (1968) found nitrite contents of 0.1 to 4.9 mg/g (30 to 1,470 ppm NO_2-N) at pH 8.0 to 8.5 in deposits in shafts of poorly ventilated houses. Experiments showed that the nitrite was produced by *Nitrosomonas* species; further, the ammonia that formed the substrate for production of nitrite prevented it from being further metabolized to nitrate.

That nitrite poisoning of livestock may not always be related to natural phenomena was demonstrated by the report of Balogh *et al.* (1965). Ammonium nitrate fertilizer was accidentally mixed with corn and then fed to 2,300 chickens. Obvious signs of methemoglobinemia were present in 800 birds. Methylene blue was effective in limiting death losses, although 102 birds died. In subsequent feeding trials with normal chickens, 3 to 4 g/kg of the contaminated feed produced methemoglobin levels of 36 to 85 percent.

NITRITE TOXICITY IN HUMANS

Most clinical cases of nitrite toxicity in humans occur in infants or young children who have consumed food or water containing elevated nitrate levels (Lee, 1970; National Academy of Sciences, 1972).

Many common table vegetables have been found to contain high levels of nitrate (Lee, 1970). Sufficient nitrate to induce nitrite toxicity in infants may be present in fresh or recently prepared vegetables or may form following improper storage (Sinios and Wodsak, 1965). If contaminated vegetables are fed after nitrite is formed and before nitrite reduction can occur, chances of toxicity are increased; this is especially true when the water in which the vegetable was prepared is consumed. Schuphan (1965) has shown that overfertilization may be responsible for high levels of nitrate in vegetables (spinach) and that nitrites may then form during transport and storage.

Methemoglobinemia may also occur in persons who have consumed preserved meats with unusually high nitrite levels. Such an instance was reported by Orgeron *et al.* (1957). Wieners containing 5,420 ppm nitrite caused illness in 10 victims less than 10 years old; all were treated successfully with methylene blue. A similar case was ascribed to consumption of kiszka, Polish blood sausage cured with nitrite to preserve a blood-red appearance (Bakshi *et al.*, 1967).

The possibility of nitrates occurring in food prepared from animals that had consumed high levels of nitrates was examined by Davison, Hansel, *et al.* (1964). The only change in milk composition associated with nitrate administration to dairy cattle was a significant but small increase in nitrate content: Milk nitrate from heifers fed 660 mg

nitrate/kg body weight contained 15 ppm nitrate; cattle ted a control ration produced milk that contained 5 ppm nitrate. Average nitrate content of meat from cattle fed nitrate-supplemented rations was 21 ppm, compared to 5 ppm in the controls. These levels seem acceptable in view of permissible levels of 200 ppm nitrite in processed meats and 45 ppm nitrate in water.

Numerous reports of methemoglobinemia in infants have appeared since Comly's observation (1945) that 64 to 140 ppm NO_3-N in water used to prepare formula milk would induce "infantile cyanosis" (see bibliographies of Burden, 1961; Lee, 1970; Singer, 1968). Through 1970, approximately 2,000 cases of methemoglobinemia in infants had been reported in North America and Europe. Winton *et al.* (1971) cite an investigator who estimates that these probably represent 10 percent of the actual cases, since the disease is not required to be reported. The last such death from excessive nitrates in water reported in the United States was in 1949.

Werner *et al.* (1965) summarized observations on 194 cases of well water methemoglobinemia in German infants. Thirty were seriously affected; eight died; others survived methemoglobin concentrations of 60 to 84 percent. The highest concentration of the nitrate encountered in water was 862 ppm. Knotek and Schmidt (1964) found that of the infants born between 1953 and 1960 in communities near Prague, Czechoslovakia, 2 percent suffered from methemoglobinemia. A greater percentage of artificially fed infants were affected in areas where the water contained high concentrations of nitrates. While only one reported case of infantile methemoglobinemia has been associated with municipal water supplies in this country (Vigil *et al.*, 1965), eight cases were associated with tap water in Paris (Aussannaire *et al.*, 1968).

Recently, a prospective epidemiological study was undertaken in southern California and central Illinois to determine the amounts of nitrate being consumed in water by infants and the minimum dose that would produce detectable methemoglobinemia (Winton *et al.*, 1971). In this study, 111 infants from less than two weeks old to six months old were observed and their drinking water analyzed. Sixty-three were exposed to less than 1.0 mg NO_3/kg body weight (0.23 mg NO_3-N/kg), 23 to between 1.0 and 4.9 mg NO_3/kg (0.23 to 1.13 mg NO_3-N/kg), 20 to 5.0 to 9.9 mg NO_3/kg (1.15 to 2.28 mg NO_3-N/kg), and five to 10.0 to 15.5 mg NO_3/kg (2.3 to 3.57 mg NO_3-N/kg). Three infants who received the highest level had methemoglobin levels above normal. The highest methemoglobin level was 5.3 percent, compared to a normal range of 0 to 2.9 percent. None of the infants demonstrated signs of methemoglobinemia. Winton estimated that an infant might obtain a

daily nitrate dose of 10 to 15 mg/kg if drinking water with a nitrate level as low as 70 ppm (16 ppm NO_3-N) were used to prepare a powdered formula.

PERMISSIBLE LIMITS OF NITRATE/NITRITE INGESTION FOR HUMANS

Drinking Water

Burden, in 1961, published approximate permissible levels for nitrate in drinking water, emphasizing the variations that might occur as a result of differences in climatic conditions and the age of the consumer. Table 3, which summarizes Burden's data, was constructed on the following assumptions:

1. Nitrate is completely reduced to and absorbed as nitrite.
2. The lethal dose of NO_3-N for humans is about 20 mg/kg.
3. One-fifth of the lethal dose, or 4 mg/kg, is the maximum daily amount of NO_3-N that can be tolerated without giving rise to toxic symptoms.

The differences between permissible levels in the tropics and in England is due to variation in fluid intake, the result of climatic conditions. The fluid intake of an infant (per kilogram of body weight) is also considerably more than that of an adult.

The current permissible level for nitrate in drinking water, as recommended by the U.S. Public Health Service (1962) and established from retrospective studies, is 10 ppm NO_3-N or 45 ppm NO_3. Retrospective studies do not accurately determine disease incidence, so the currently recommended level is only an approximation of a level below which infant methemoglobinemia would be highly unlikely. As Winton *et al.* (1971) concluded, "At this time, however, there is insufficient evidence to permit raising the recommended limit, and there are some indications that the current recommendation may offer the respectable safety-factor needed to cover all reasonable situations."

Table 3. Permissible limits for nitrate nitrogen in drinking water

Consumer	Assumed weight (kg)	Region	Maximum permissible dose NO_3-N (mg)	Approx. daily water intake (liters)	Permissible nitrate limit in water (ppm)	
					N	NO_3
Infant	3	England	12	0.5	24	106
Infant	3	Tropics	12	2	6	26
Adult	60	England	240	1	240	1,056
Adult	60	Tropics	240	5.3	45	198

Food

Acceptable levels of nitrate and nitrite in foods intended for human ingestion were published by the Joint FAO/WHO Expert Committee on Food Additives in 1962. The report of this committee indicated that daily consumption of sodium nitrate at 5 to 10 mg/kg body weight or sodium nitrite at 0.4 to 0.8 mg/kg were levels at which no significant toxicological effect would be likely to occur in man.

These levels do not apply to infants, however, since persons under six months of age are characteristically more susceptible to methemoglobinemia. Preparing food to be consumed by infants presents a special problem. Such food should be prepared hygienically and stored for only short periods after having been exposed to nitrate-reducing microorganisms. Since milk formulae constitute the major component of the infant's diet, water containing more than 45 ppm nitrate is considered unsafe, according to the current U.S.P.H.S. standard.[1]

Permissible levels for nitrate and nitrite are 500 ppm and 200 ppm, respectively, in processed meat (U.S. Department of Agriculture, 1973) and fish products, with further limitations on some smoked fish products (U.S. Food and Drug Administration, 1974). In light of the recent demonstrations of *in vitro* and *in vivo* nitrosamine formation from nitrite and secondary amines, the permissible levels of nitrite additives should be reexamined (National Academy of Sciences, 1972). Long-term feeding experiments should be conducted to evaluate possible formation and effects of a variety of nitrosamines in animals with gastric pH similar to that of man.

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[1] It should be noted that the standard is set at the low level of 45 ppm in order to avoid any possible risk to infants. There may well be, however, suitable alternatives that would be preferable to disrupting food production methods in some areas in order to meet the standard. The Center for the Biology of Natural Systems, Washington University, in its annual report for 1972 (unpublished) states that vitamin C dosages at levels routinely prescribed by pediatricians are adequate to prevent methemoglobinemia. Gruener and Shuval add, "... there are certain nutrients such as vitamin C that can cure or prevent methemoglobinemia. High vitamin C intake among infants in some areas may explain the scarcity of the disease even when waters rich in nitrates are consumed" ("Health aspects of nitrates in drinking water," in *Developments in water quality research*, H. I. Shuval, ed., Ann Arbor-Humphrey Science Publ., 1970; p. 96). Reduction of methemoglobin by glutathion and ascorbic acid is discussed by Lemberg and Legge in *Hematin compounds and bile pigments* (Interscience, New York, 1949; p. 522). Using other sources of water for infant formulae may also be a suitable protective measure. — *Samuel R. Aldrich, Assistant Director, Illinois Agricultural Experiment Station.*

SUMMARY AND CONCLUSIONS

There appears to be sufficient evidence in the literature to support the view that nitrate, nitrite, and nitrosamines constitute a potential hazard to the health of humans and other animals. Nitrite toxicity in human beings has been limited almost exclusively to infants fed formulae prepared with water from rural wells that contain nitrates in excess of standards recommended by the U.S. Public Health Service. The last reported human death from nitrite toxicosis in the United States was in 1949. Of less frequent occurrence is methemoglobinemia that may develop following consumption of processed meats containing unusually high nitrite levels. More recently, nitrosamines have been identified as potentially hazardous to human health. However, no evidence has been obtained thus far to indicate that foods consumed by persons in the United States contain sufficient levels of nitrosamines to induce carcinogenic, mutagenic, or teratogenic effects.

Numerous studies have been reported confirming earlier clinical observations that nitrates and nitrites may produce acute nitrite toxicity in livestock. Signs of acute toxicity most commonly follow consumption of feeds that have accumulated excessive nitrates as a result of environmental factors or feeds to which high levels of nitrate or nitrite have been added accidentally. A large volume of data exists on what levels of nitrates and nitrites produce nitrite toxicity in animals. It is inappropriate, however, to set standards on the basis of these data because of the variations in experimental design and the possibility of significant interspecies variation as well as variations within a particular species that are related to diet, metabolic status, and age.

Conversely, attempts to experimentally reproduce the chronic or sublethal effects in livestock (effects attributed to long-term ingestion of low levels of nitrate or nitrite) have generally been unsuccessful. Under adequate maintenance and moderate temperatures, ruminants do not develop thyroxine deficiency from near-lethal levels of nitrate in the feed. Dietary nitrate may interfere with thyroxine synthesis in other species, but the effect appears to be transitory. Although prolonged ingestion of nitrite may lead to avitaminosis A in monogastric animals, this condition appears unlikely to develop in ruminants because of the near neutral pH of ruminal contents.

Production losses and lower weight gains in animals that consume feed or water containing high levels of nitrate or nitrite do not appear to be due to direct toxic effects of nitrate or nitrite but rather to the poor palatability of the feed or water and hence decreased consumption.

Finally, experimental evidence has shown that nonspecific abortions cannot be explained by ingestion of sublethal levels of nitrate or nitrite.

The following conclusions may be drawn from information presented in this review:

1. Nitrate content of water is a matter for public concern because of the potential health hazard for infants. Efforts should be continued to identify drinking water sources that contain nitrates in excess of the standard recommended by the U.S. Public Health Service and to publicize procedures that will protect infants. (The U.S.P.H.S. standard has been and will likely continue to be reevaluated. Compliance with the standard would require some drastic changes in food production practices in some areas. Methods for protecting infants are readily available — using alternative sources of water, for example.) Drinking water has seldom if ever been implicated in naturally occurring nitrite toxicosis of livestock.

2. Although human infants are far more susceptible than adults to methemoglobinemia, young cattle and swine do not appear to be any more susceptible to methemoglobinemia than mature animals.

3. There is little evidence to indicate that nitrosamines are more than a potential hazard to animal or human health. However, long-term feeding experiments should be conducted with animals with gastric pH similar to that of man to evaluate possible formation and effects of nitrosamines.

4. The acute effects of nitrates and nitrites in livestock are well documented. But attempts to reproduce chronic effects attributed to nitrates and nitrites have generally been unsuccessful. Thus, until sufficient experimental evidence is available to relate long-term consumption of sublethal levels of nitrate or nitrite to chronic effects, reports of such effects should be evaluated with caution.

5. Nitrite toxicity may occur when animals consume forages that have accumulated nitrates as a result of environmental factors; however, dangerous accumulations of nitrate in forages have not been correlated with efficiently used nitrogen fertilizers.

6. Well-documented herd studies of naturally occurring nitrite intoxication are rare. Definitive field studies of livestock herds in which nitrite intoxication is suspected would provide valuable information for assessing the true significance of nitrite toxicity in livestock.

7. When cases of methemoglobinemia are suspected in livestock, every effort should be made to substantiate the diagnosis. Methemoglobin and nitrate/nitrite levels of body fluids of affected animals should be determined, and suspected feed or water should be analyzed for nitrate/nitrite content.

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